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*Kidney International, Vol. 68, Supplement 99 (2005), pp. S108–S112*



# Longitudinal analysis of chronic allograft nephropathy: Clinicopathologic correlations

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**Longitudinal analysis of chronic allograft nephropathy: Clinicopathologic correlations.**

*Background.* Loss of the allograft from chronic allograft nephropathy and death of the patient from vascular, malignant, or infective disease are the major problems in renal transplantation today. Protocol biopsy of the long-term kidney has provided new data with which to develop strategies for prevention and treatment of chronic allograft nephropathy.

*Methods.* Two series of long-term protocol biopsies are reviewed. In the first, renal biopsies were obtained at time 0, and at 3 months and 12 months, and the recipients of the renal allografts were followed up for up to 15 years. In the second, the kidneys of recipients of simultaneous pancreas kidney transplants were biopsied annually for 10 years, and the results correlated with clinical events.

*Results.* Chronic allograft nephropathy is caused by acute and chronic immune-mediated damage, as well as by chronic calcineurin inhibitor nephrotoxicity. Both immune and nonimmune mechanisms exacerbate pre-existing donor disease and ischemia-reperfusion injury. Established interstitial fibrosis and arteriolar hyalinosis lead to progressive glomerular sclerosis and eventual loss of the graft.

*Conclusion.* Protocol biopsies have shown that clinical parameters of renal function underestimate the severity of chronic graft damage. Strategies for preventing or treating chronic renal allograft dysfunction and subsequent graft loss must better control rejection and simultaneously avoid nephrotoxicity.

Chronic allograft nephropathy (CAN) is the cause of a majority of graft failures now that the risk of acute allograft rejection has diminished [1]. Although the issue is quite hotly debated, it is clear that the reduction in incidence of acute rejection has had little or no effect on the long-term prognosis of grafts [2]. Patient death from vascular, malignant, or infective disease and loss of the allograft from chronic allograft nephropathy are the major problems in renal transplantation today [1, 3].

Understanding the phenomenon of "chronic rejection" or "chronic allograft nephropathy" is thus central to our ability to tackle the long-term attrition of grafts. The kidney has a relatively restricted response to injury, and so a simple description of the histological appearance at any particular point in time may not help in understanding causality. However, data from a number of longitudinal histological studies are beginning to unravel these relationships and thus assist not only in understanding the processes of chronic allograft damage, but also in identifying prevention and treatment strategies.

**Key words:** renal transplant, chronic allograft nephropathy, renal dysfunction, nephrotoxicity, calcineurin inhibitors.

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## CLINICAL FEATURES OF CAN

Factors that relate to the donor, transplant surgery, and the recipient determine the initial function of a renal transplant, which varies widely between patients. The terms "intercept" and "slope" have been used to describe the different influences on renal allografts as they deteriorate and fail [4]. The kidney from a young living donor who suffers no early acute rejection may yield an initial glomerular liltration rate (GFR) of 70 mL/min, which may rise to 100 mL/min in the early months because of glomerular hyperfiltration. By contrast, the kidney from an elderly deceased donor with a history of hypertension and 20% sclerosed glomeruli on the implantation biopsy, which then suffers from significant ischemic damage and early rejection, may only achieve a maximum GFR of 30 mL/min. The "intercept" values are thus 100 mL/min and 30 mL/min, respectively. Even if the subsequent "slope" of decline in GFR is 5 mL/min/year identically for both kidneys, on clinical follow-up they will appear to behave very differently in the next years. The GFR will reach 10 mL/min in only 4 years for the damaged kidney, but it will take 18 years for the undamaged kidney to reach the same level. The pathophysiology of the "slope" may be the same in the two instances, but the clinical effect is dramatically different.

The poorly functioning graft will have dropped the initial serum creatinine level to perhaps only 180 μmol/L. Within a year, the creatinine will reach 220 μmol/L, but may have fluctuated between 170 μmol/L and 220 μmol/L during the year, as the variable influences of acute

calcineurin inhibitor (CNI) toxicity, hydration status, and concomitant infections have impacted on the patient. The alarm will be raised as the creatinine rises from 220 μmol/L to 250 μmol/L or higher, and a biopsy may or may not be performed. Proteinuria and hypertension will be noted at this point in the follow-up, and angiotensin-converting enzyme inhibitors may be commenced. If the biopsy had been performed, the pathologist would have reported grade III CAN. A clinical diagnosis of CAN is determined in the absence of a biopsy, and a series of therapeutic strategies may be implemented with varying levels of success.

The younger living donor kidney is regarded as a huge success with a starting GFR of 100 mL/min. The GFR of this kidney also falls at a steady 5 mL/min each year, but there is no impact on the creatinine value, which is stable between 80 μmol/L and 90 μmol/L. It is only in the 14th year of the transplant that the creatinine level begins to cause concern, because the GFR has fallen to 30 mL/min, and the creatinine is now noticeably higher than it was a few years ago. The surgeon and physician who performed the transplant in the patient have retired, and it becomes apparent to the patient that their care was excellent, because the kidney only started to deteriorate when the new physician arrived. The new physician biopsies the kidney, the pathologist reports grade III CAN, and the young physician privately blames his predecessors for not diagnosing and managing the problem earlier. The chronic pathologic process has been identical in the two kidneys, but the impact of the renal functional reserve has differentiated the two clinical courses.

## THE PATHOLOGIC FEATURES OF CAN

Classification of renal transplant histology was given the necessary intellectual and physical impetus through a series of meetings in Banff [5–8], which focused initially on acute rejection. "Chronic allograft nephropathy" (CAN) was defined histologically, with those words deliberately chosen to avoid the implications of etiology carried by the words "chronic rejection" [5]. However, Colvin has noted that identification of specific features can lead to an etiologic assignment in around 60% of all biopsies [9]: e.g., interstitial fibrosis and tubular atrophy with nodular arteriolar hyalinosis implying CNI toxicity; and vascular changes with disruption of the elastica, inflammatory cells in the fibrotic intima, and proliferation of myofibroblasts in the intima implying chronic immune-mediated rejection.

CAN is defined and graded in the Banff 97 system [5] by identification of interstitial fibrosis and tubular atrophy. These features were selected because they are widespread and relatively reproducible in small tissue samples. CAN is graded from I to III based on the proportion of the cortical area of the biopsy affected by chronic interstitial fibrosis and tubular atrophy (grade I = 6%–25%, II = 26%–50%, III = >50%). Linkage between the CAN grade and the chronic Banff qualifiers for interstitial fibrosis (ci) and tubular atrophy (ct) is imprecise, because the overall appearance of the sample is graded directly for CAN and not indirectly from the severity of its components. Common usage has lead to the term CAN being a description and grading of chronic damage to the graft, whether it has resulted from prior acute rejection, calcinerin inhibitor nephrotoxicity, hypertensive nephrosclerosis, or some other etiology.

Modern biopsy techniques make the acquisition of histology data much safer, with the routine use of ultrasound localization, 18 G needles, and spring-loaded biopsy guns. A multicenter European study of 2127 biopsies demonstrated one attributable graft loss and three direct interventions for bleeding [10]. The risk-benefit ratio for the individual patient has swung to favor protocol-driven biopsy schedules rather than simply be event-driven, which leads to a sound basis for examination of the underlying behavior of the disease processes leading to CAN.

## LONGITUDINAL HISTOLOGY RESULTS

Our group in Sydney has published two series of protocol biopsies in which we have addressed the causes and correlates of CAN, the statistically significant results of which are reported below. In the first series, biopsies were obtained at time 0 and at 3 months and 12 months from recipients of renal allografts. These patients were then followed up clinically for up to 15 years [11, 12]. In the second series, recipients of simultaneous pancreas kidney transplants were biopsied annually for 10 years, yielding approximately 1000 biopsies from 120 patients [13–17]. The two series have different attributes, especially with respect to the types and ages of the donors from which the kidneys were derived. The first series included kidneys from deceased and living donors of all ages with a variety of pre-existing conditions and was able to demonstrate the correlates and predictive value of early damage. In contrast, the kidneys in the second series were all transplanted with short ischemia times from donors under 45 years of age, with almost no histological abnormalities on the day 0 biopsy. This series is thus powerfully able to identify the histological evolution of long-term graft fibrosis [13] and describe the natural history of CAN in the kidneys of diabetics receiving simultaneous pancreas transplants. A strength of the study has proven to be the unveiling of the intrarenal relationships between fibrosis and arteriolar and glomerular damage.

## PREDICTORS OF 3-MONTH AND 12-MONTH HISTOLOGY

The 3-month protocol biopsy histology revealed both chronic and acute changes that were characterized using

the then-current Banff schema [11, 12]. At 3 months, only 22% of grafts were normal and 54% had CAN grade I, whereas the remaining 24% already had CAN grade II or III. The chronic changes were predominantly interstitial fibrosis and tubular atrophy, together with chronic vascular disease. The statistically significant predictors of interstitial disease were donor age, prior vascular rejection, and delayed graft function. Vascular disease was predicted by donor vascular disease, total cold ischemic time, and prior vascular rejection. The histology at 12 months correlated with changes on the 3-month biopsy. Acute Banff qualifiers in the 3-month biopsy were: interstitial infiltrate, tubulitis, and vasculitis (i, t, and v, respectively), each correlated with the relevant chronic changes in the 1-month biopsy (ci, ct, cv). Thus, for each individual patient, subclinical rejection detected on the 3-month protocol biopsy led to the development of CAN by 12 months.

Renal functional decline and ultimate graft survival were predicted by the 3-month ci and cv scores, as well as by the presence of proteinuria. Late acute rejection, presumed in most cases to result from noncompliance, was also a strong predictor of late graft loss. Indices of cyclosporine exposure, the presence of hypertension, and donor and recipient age did not, however, correlate with outcomes.

## LONG-TERM PROTOCOL HISTOLOGY

The second series of protocol biopsies was more extensive than the first. The biopsies were taken from 1987 to 1999 and were performed as part of a long-term study of the impact of simultaneous pancreas and kidney transplantation in patients with insulin-dependent diabetes mellitus [13]. The follow-up protocol included measured GFR, an oral glucose tolerance test, renal transplant biopsy, and recording of clinical parameters at each time point. The overall 1-year survival was excellent, with 96% patient survival, 94% kidney survival, and 85% pancreas survival. Similarly good results were seen for the 10-year survival of patients (80%), kidneys (77%), and pancreases (67%) [13]. The average measured GFR of the surviving kidneys was 61 mL/min at 1 year and 50 mL/min between 6 and 10 years. In the context of these good results, the pathologic changes were surprising and disappointing, but correctly predicting graft failure for several long-term patients between 10 and 15 years.

## SUBCLINICAL REJECTION

Subclinical rejection was defined as either borderline/suspicious or acute subclinical rejection in patients with stable serum creatinine values at the time of biopsy, using the histological hallmarks of rejection (Banff i and t scores) [14]. The frequency of interstitial inflammation and tubulitis declined exponentially with time after transplantation; was dependent on the immunosuppressive protocol, being less common with tacrolimus than cyclosporine, and mycophenolate mofetil than azathioprine; and was persistent in only 6% of patients. The impact of both borderline and acute subclinical rejection was to increase the subsequent incidence of interstitial fibrosis and tubular atrophy, mimicking the data in the first series. Persistent subclinical rejection, proposed as the definition of true chronic cellular rejection, not only increased graft fibrosis but also reduced the GFR by about half over 2 years. Chronic inflammation, restricted to areas of interstitial fibrosis and thus ignored under the Banff schema, was also seen to correlate with increased fibrosis in follow-up biopsies.

Immunosuppression was clearly effective in reducing both subclinical rejection and preventing its sequelae, with micro-emulsion cyclosporine (Neoral; Novartis, Basel, Switzerland) being more effective than the older formulation (Sandimmune, Novartis); and with tacrolimus (Prograf, Astellas, Tokyo, Japan) being more effective than cyclosporine in reducing the density of cellular infiltrate. Mycophenolate mofeil reduced tubulitis, while the combination of tacrolimus and mycophenolate mofetil effectively abolished subclinical rejection by 3 months. Tapering of the corticosteroid dose between 3 and 6 months led to an increase in the prevalence of subclinical rejection in cyclosporine-treated patients.

## INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY

Mild fibrosis was present in only 5% of implantation biopsies in this series of young donors selected for the suitability of the pancreas. Both interstitial fibrosis and tubular atrophy increased markedly during the first year and thereafter accumulated at a slower rate [15]. Two thirds of the total burden of fibrosis occurred in the first year and was associated with acute tubular necrosis, acute rejection, and untreated subclinical rejection. Interstitial fibrosis exceeded loss from tubular atrophy 2-fold in the first year, but thereafter tubulointerstitial injury developed simultaneously. As the level of mononuclear cell infiltration declined with time as a cause of fibrosis, the importance of CNI toxicity increased. Tubulointerstitial damage preceded and correlated with the degree of glomerulosclerosis observed in subsequent biopsies. Renal function, as expected, declined in proportion to the amount of fibrosis, from a measured GFR of 65 mL/min in patients with normal biopsies to 59 mL/min and 44 mL/min with mild and moderate fibrosis, respectively. Once established, tubulointerstitial damage did not regress in subsequent biopsies. Immunosuppression considerably modified the degree of damage seen, mediated predominantly through the different impacts of the drugs on acute, subclinical, and persistent rejection.

## ARTERIOLAR HYALINOSIS AND CNI NEPHROTOXICITY

CNI nephrotoxicity has been defined histologically through presence of the characteristic lesions of nodular arteriolar hyalinosis, striped interstitial fibrosis, and tubular microcalcification. These lesions were detected in 100%, 88%, and 79% of patients in this series by 10 years [16]. At 1 year, 54% of patients had two of these histological hallmarks present, rising to 100% by 10 years. A threshold cyclosporine dose of 5 mg/kg/day was associated with development of CNI nephrotoxicity within the first 5 years. Although classical striped fibrosis and nodular hyalinosis are largely restricted to CNI nephrotoxicity, diffuse arteriolar hyalinosis, tubular calcification, and the latter stages of diffuse interstitial fibrosis have a number of potential etiologies. In this study, arteriolar hyalinosis was correlated with measures of cyclosporine exposure, including trough levels greater than 200 ng/mL at 3 months and preceding episodes of acute reversible nephrotoxicity. There was, however, no correlation with oral glucose tolerance or glycosylated hemoglobin. Hypertension preceded arteriolar hyalinosis and was present in 68% of hypertensive and 60% of normotensive patients. The appearance of de novo diffuse arteriolar hyalinosis was thus unrelated to blood pressure, dyslipidemia, glucose tolerance, or ischemia, and was specific for CNI nephrotoxicity. There were no differences between tacrolimus and cyclosporine in the histological measures of CNI nephrotoxicity, which supports data from other studies [18].

## GLOMERULOSCLEROSIS

First demonstrated in 1963 [19], transplant glomerulopathy is characterized by enlarged glomeruli, mesangial matrix expansion, changes in mesangial cells, and splitting of the glomerular basement membrane. It was uncommon in our series of unsensitized first graft recipients, as was evidence for recurrence of diabetic nephropathy. Chronic and progressive glomerulosclerosis, by contrast, was common and occurred in two phases [17]. The early phase of glomerular damage, seen within the first month, was associated with duration of cold ischemia and acute CNI nephrotoxicity. The later and more important phase of progressive glomerulosclerosis occurred after 4 to 5 years, beyond which time one third of all glomeruli had been lost. The twin causes of glomerular loss were preceding tubulointerstitial nephritis and arteriolar hyalinosis. The 1-year Banff chronic interstitial fibrosis score and prior subclinical rejection correlated with development of glomerulosclerosis by 5 years. Grafts with no interstitial fibrosis at 1 year also eventually developed glomerulosclerosis, but to a lesser intensity. Arteriolar hyalinosis leading to glomerular ischemia was the second major explanation for glomerular loss. A Banff score of ah2 or above in one biopsy led to an increase in glomerulosclerosis in the subsequent biopsy. The degree of arteriolar hyalinosis also correlated strongly with the percentage of sclerosed glomeruli in the same biopsy, with a 3-fold rise between biopsies with ah1 and ah3. Glomerulosclerosis correlated significantly but poorly with GFR, unlike the much tighter correlation with tubulointerstitial damage. GFR fell in parallel with the increase in glomerulosclerosis, but underestimated the degree of damage, falling from 59 mL/min with no glomerulosclerosis to 56 mL/min with 1% to 20% and 52 mL/min with more than 20% of glomeruli sclerosed.

## CONCLUSION

CAN has two primary causes. First, it results from immune-mediated damage either from acute severe or vascular rejection, or from undiagnosed and thus untreated subclinical rejection, commonly present early and uncommonly present persistently in later years. Second, CAN results from CNI nephrotoxicity, which causes arteriolar hyalinosis and interstitial fibrosis. Both immune and nonimmune mechanisms exacerbate pre-existing donor disease and the ischemic insult that all grafts suffer to a varying extent. The establishment of interstitial fibrosis and arteriolar hyalinosis independently and together causes progressive glomerular sclerosis, which precedes decline in the GFR. Clinical programs monitor change in the serum creatinine to detect patients at risk of CAN, but the protocol biopsy evidence now shows that such clinical parameters dramatically underestimate the severity of chronic graft damage. Strategies for intervening to prevent chronic renal allograft dysfunction and subsequent graft loss need to better control rejection and, at the same time, simultaneously avoid the nephrotoxicity that we have been living with, and accepting, for the past 20 years.

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